A STUDY ON PROTEIN OXIDATIVE DAMAGE IN MICE INDUCED BY GASEOUS FORMALDEHYDE

LJ Duan, Y Zhu, QL Hu, YS Liu, QP Qi, X Yang *

College of Life Science, Central China Normal University, Wuhan 430079, China

ABSTRACT
To explore protein oxidative damage induced by gaseous formaldehyde and its mechanism we carried out this study. Mice were treated with gaseous formaldehyde at different levels (0mg.m^{-3}, 0.68mg.m^{-3} and 3.0mg.m^{-3}) for 72 hr and the protein carbonyl content was mensurated using spectrophotometric DNPH assay to reflect the degree of protein oxidative damage. The results show that the protein carbonyl content of brain, heart and liver tissue in mice decrease in the group of 0.68mg.m^{-3} ($p<0.01$, $p<0.01$, $p<0.05$), while increase significantly in heart and liver tissue in the group of 3.0mg.m^{-3} ($p<0.01$, $p<0.01$) and protein carbonyl content in brain does not have notable difference compared with the control ($p>0.05$). These results indicate that the protein oxidative damage induced by gaseous formaldehyde depends on the concentration of gaseous formaldehyde. Medium concentration formaldehyde may not induce protein oxidative damage, while high concentration formaldehyde can cause protein oxidative damage in heart and liver tissue markedly and has little effect on protein of brain.

INDEX TERMS
Formaldehyde; Protein oxidative damage; Carbonyl content

INTRODUCTION
Oxidative damage of formaldehyde to cell is mainly due to restrain function of formaldehyde to anti-oxidation system. It can make free radicals accumulate in economy and can not be cleaned up in time. This can result in extensive harmful effect to economy. The previous studies of our group investigated the effects on anti-oxidative system and LPO induced by gaseous formaldehyde in some apparatus. Results showed that after 3mg.m^{-3} formaldehyde exposure the enzymatic activities of SOD, GSH-PX and CAT deceased, while GSH content decreased and MDA content increased. Yang Dan-feng et al also investigated the effects of gaseous formaldehyde on anti-oxidative system of rats. The results showed that 13.5mg.m^{-3} formaldehyde made GSH content and activity of GSH-PX reduced and MDA content increased in blood (YANG Dan-feng, 2004). However, only using these indexes to evaluate the degree of oxidative damage is not comprehensive, because these biomarkers can not reflect the direct effect of formaldehyde on proteins, which take on important physiological function in the body. Some literatures reported that oxidation of side chain of protein amino acid can cause accumulation of carbonyls. Carbonyl to protein is widely used to evaluate oxidative degree in all kinds of biology organism (Cederberg J 2001) and protein carbonyl is a sensitive biomarker for protein oxidative damage (Stanley T et al 2000). Therefore, to search for the direct evidence of protein oxidation induced by air formaldehyde we undertook this investigation.

METHODS
Materials And Instruments
Materials of study are SPF class Kun Ming male mice provided by the medicine experiment animal center of Hubei province. The age is 5 weeks and the weight is about 25g. Main instruments: small scale environmental chamber for generating gaseous formaldehyde, formaldehyde analyzer, centrifuge, water bath, 751-spectrophotometer and vortex.

Main Reagents
Aprotinin, Sigma company; PMSF and carbamidine hydrochloric acid, Amresco company; Streptomycin sulphate, Duchefa company; Kit of reagent of detection (coomassie brilliant blue), Nanking Jiancheng; KCl, NaCl, KH_2PO4, $MgSO_4$, EDTA, DNPH, ethanol, TCA and HCl are all reagents made in China.

Treatments With Formaldehyde
Environmental exposure mode (LI R. 2003) was adapted in these experiments. Fifteen mice were randomly

* Corresponding author email: yangxu@mail.ccnu.edu.cn, guyangxu@yahoo.com

divided into 3 groups. One was the control group and the other two were exposure groups. Each group had five mice. The mice of exposure groups were continuously exposed to gaseous formaldehyde for 72hr in a small glass chamber (8.4L). Formaldehyde concentration was kept at 0.5 $mg.m^{-3}$ and 3.0 $mg.m^{-3}$. The mice of control group inhaled filtrated fresh air (FA＜0.01 $mg.m^{-3}$) in the small glass chamber. In the course of exposure, animals were allowed to eat freely.

Gaseous formaldehyde used in exposure was prepared by using an emulational way. We placed some woody artificial boards in WH-2 small scale environmental chamber, adjusted the amount of boards, and made environmental chamber release steady-going gaseous formaldehyde. Parameters of chamber was that chamber air temperature was (23±0.5)℃, air humidity was 45% and gas runoff was 1L.min^{-1}. Concentration of gaseous formaldehyde was measured by using 4160-2 formaldehyde analyzer. Its sensitivity was 0.012 $mg.m^{-3}$.

Tissue Sample Preparation

After exposure, the mice were killed. Their brains, hearts and livers were collected. Tissue (100mg) was homogenized in 1mL of homogenizing buffer in a 1mL glass homogeniser. The homogenized tissue was transferred to a plastic tube and a 0.1 $g.mL^{-1}$ streptomycin soleplate solution was added to a final concentration of 0.01g. mL^{-1}. The solution was mixed and left to stand at room temperature for 10 minutes. It was then centrifuged for 10 minutes and the supernatant was removed, (Nukada H & Mcmorran PD 1999).

Assay Of Tissue Protein Carbonyl Content

Principle of spectrophotometric DNPH assay: Spectrophotometric DNPH assay was used to measure protein carbonyl content in experiment. It is a classical method used to measure protein carbonyle content. When the protein is oxidized, its carbonyl content increased. Carbonyl can have a reaction with 2,4-dinitrophenylhydrazine and create DNPH-derivatised proteins which is red depositions. The red depositions are dissolved with guanidine hydrochloride, (Nukada H &Mcmorran PD 1999).

Statistics Analysis

Data were tested by Origin 4.1 software. The differences between control and exposure groups were undertaken by t-test.

RESULTS

Exposure Doses

Gaseous formaldehyde concentrations were measured three times each day. Formaldehyde concentration of control group was 0$mg.m^{-3}$. The concentrations for exposure groups were 2.97±0.19$mg.m^{-3}$ (planed value was 3.0$mg.m^{-3}$) and 0.68±0.10$mg.m^{-3}$ (planed value was 0.5$mg.m^{-3}$), which was more than 0.5 $mg.m^{-3}$.

Dose/Response Effects

Table 1 showed the results. The dose/response effect between formaldehyde concentrations and protein carbonyl content is good. From table 1 we can see that compared with the control, carbonyl content of brain and heart tissue significantly decreases ($p<0.01$) and carbonyl content of liver decreases ($p<0.05$) when concentration of gaseous formaldehyde is 0.68$mg.m^{-3}$. Compared with the control, carbonyl content in brain tissue has no significant difference ($p>0.05$), while carbonyl content of heart and liver significantly increased when concentration of gaseous formaldehyde was 3.0 mg m^{-3} ($p<0.01$).

Table 1. *Relation between formaldehyde concentrations and protein carbonyl content in mice* ($n=5$, $\bar{X}\pm S$, $nmol.mg^{-1}$)

treatment	brain	heart	liver
control	20.89±4.37	10.34±1.69	20.39±3.92
0.68mg.L-1	5.75±2.39**	3.82±0.46**	15.04±2.00*
3.0mg.L-1	19.87±5.19	45.5±22.49**	59.44±29.02**

DISCUSSION

Now Chinese National Occupational Health Standard for formaldehyde is 0.5$mg.m^{-3}$. In our study 0.68$mg.m^{-3}$ gaseous formaldehyde can not make protein carbonyl content increase. This may support that low concentration gaseous formaldehyde can not induce obvious oxidative damage to proteins and, for this view point, the health standard may be safe enough.

Effect of medium concentration of air formaldehyde to protein of body: 3.0mg.m^{-3} gaseous formaldehyde make protein carbonyl content of heart and liver tissue significantly increased. Medium concentration of formaldehyde may damage proteins. Its molecular mechanism likes that the overload formaldehyde directly inhibits the activity of enzyme of anti-oxidative system, which can make excessive free radical accumulate in economy and can not be eliminated in time (WEN Jing et al 2002). The other possible mechanism is that excessive formaldehyde is metabolized in body and it makes excessive free radicals. By the effect of MCO system, free radicals attack amidocyanogen and sub-amidocyanogen in proteins and finally create carbonyle ramifications (WEN Jing et al 2002). Proteins which have more carbonyls are easy to make crosslink, assemble to big molecules and decrease function of proteins. This kind of proteins aggravate the burden of protein hydrolyze enzyme and lead to diseases (WEN Jing et al 2002).

Medium concentration gaseous formaldehyde can not make protein carbonyl content significantly increased in brain, which may relate to special protection mechanism. Meanwhile, heart and liver are sensitive apparatus and they should be used to estimate oxidative damage induced by some chemicals.

CONCLUSION AND IMPLICATIONS

0.68mg.m^{-3} gaseous formaldehyde can not induce protein oxidative damage in mice, while 3.0mg.m^{-3} gaseous formaldehyde make protein carbonyle content of heart and liver tissue significantly increased. This indicates that medium concentrate formaldehyde can result in protein oxidative damage in mice.

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